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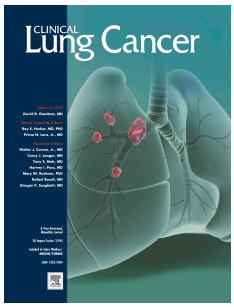

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Molecular and Immune Biomarker Testing in Squamous-Cell Lung Cancer: Impact of Current and Future Therapies and Technologies

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Abstract

Patients with non–small-cell lung cancer, including squamous-cell lung cancer (SqCLC), typically present at an advanced stage. The current treatment landscape, which includes chemotherapy, radiotherapy, surgery, immunotherapy, and targeted agents, is rapidly evolving, including for patients with SqCLC. Prompt molecular and immune biomarker testing can serve to guide optimal treatment choices, and immune biomarker testing is becoming more important for this patient population. This review provides an overview of current and emerging practices and technologies for molecular and immune biomarker testing in advanced non–small-cell lung cancer, with a focus on SqCLC.

Keywords: Non–small-cell lung cancer; Squamous-cell lung cancer; Molecular testing; PD-L1; Pathology; Immune-oncology; Biomarker; Targeted treatment

Introduction

Over the past decade, determining the histology of non–small-cell lung cancer (NSCLC) has become standard as treatment options vary by tumor histologic subtype. Multiple guidelines, including the National Comprehensive Cancer Network (NCCN), European Society for Medical Oncology (ESMO), and the College of American Pathologists (CAP)/International Association for the Study of Lung Cancer (IASLC)/Association for Molecular Pathology (AMP) guidelines, provide recommendations for performing molecular testing to further guide treatment with targeted therapies in advanced NSCLC, including squamous-cell lung cancer (SqCLC).[1-3] Immune testing, performed by immunohistochemistry (IHC), for expression of programmed death-ligand 1 (PD-L1) as a predictive marker of response to anti–programmed death-1 (PD-1)/–PD-L1 checkpoint inhibitors is also now being incorporated into many guidelines.[2, 3]

The majority of patients (68-79%) with lung cancer present at an advanced stage,[4-7] and often, only small biopsy or cytology samples are available for diagnosis.[7, 8] Therefore, it is important to prioritize biopsy tissue from NSCLC tumors to allow for use in both pathologic diagnosis and molecular and immune biomarker testing to help guide individualized treatment decisions. Herein, we review the current evidence and practice for pathologic diagnosis and molecular and immune biomarker testing in NSCLC, with a focus on SqCLC, and we evaluate how changes in the treatment and technological landscape are likely to impact molecular and immune biomarker testing in SqCLC within the next 5 years and the challenges that must be overcome.

Current Practice for Pathologic Diagnosis and Molecular and Immune Biomarker Testing in NSCLC, including SqCLC

As distinguishing between the different NSCLC subtypes has become central to patient management due to their therapeutic implications, it is recommended that samples showing NSCLC be subject to pathologic diagnosis with histologic subtyping.[9] Furthermore, current best practice involves a multidisciplinary team approach to coordinate tumor tissue optimization for both pathologic diagnosis and molecular testing to accelerate diagnostic molecular and immune biomarker testing results and to ensure that the most appropriate treatment choice is recommended to the patient in an expeditious fashion[7] (Figure 1).

The pathologic diagnosis of NSCLC subtypes, which include SqCLC, adenocarcinoma, and large-cell carcinoma, is a multistep process.[9] In most cases, the classic histologic features of tumor cells from SqCLC and other subtypes can be readily distinguished by evaluating tissue sections stained with hematoxylin and eosin.[7, 9] In the roughly 20-40% of challenging cases in which the NSCLC subtype cannot be determined by histology alone,[10, 11] limited IHC on tissue sections to specifically detect p40/p63, thyroid transcription factor-1 (TTF-1), and in a few cases, neuroendocrine biomarkers such as neuron-specific enolase and chromogranin A can be used to differentiate between SqCLC, adenocarcinoma, and large- and small-cell carcinoma, respectively.[7, 12-16] P40 is a more specific and sensitive marker for SqCLC than p63 (p40: sensitivity 100%, specificity 98%; p63: sensitivity > 90%, specificity about 60-75%), while the TTF-1 marker has > 80% sensitivity and 97% specificity for adenocarcinoma.[9, 12, 13, 17, 18] Cytokeratin 7 is preferentially expressed in adenocarcinoma[19] and can be used as a biomarker to support the diagnosis of adenocarcinoma, but only when used alongside other markers since it is not specific for adenocarcinoma.

Looking specifically at SqCLC, routine molecular testing for alterations such as epidermal growth factor receptor (*EGFR)* mutations, anaplastic lymphoma kinase *(ALK)* gene rearrangements, and *ROS* proto-oncogene 1 (*ROS-1)* gene fusions is not recommended due to their very low incidences in SqCLC (< 4% , < 3%, and 0%, respectively).[20-27] However, molecular testing for these alterations should be considered for patients with SqCLC who are younger, who have never smoked or are former very light smokers (i.e., < 15 packs per years), or for patients with small biopsy samples or mixed histology,[1-3] and potentially for patients who are of Asian ethnicity, although the latter characteristic is not included in current guidelines. The NCCN and CAP/IASLC/AMP guidelines also advise performing broad molecular testing beyond *EGFR* mutations and *ALK* and *ROS-1* gene alterations to assist in the identification of rare genomic drivers for which effective therapy may already be available (e.g., translocations of the rearranged during transfection [*RET*] gene and mesenchymal-epithelial transition exon 14 mutations) and to counsel patients regarding available clinical trials.[1, 3] With the recent approval of dabrafenib plus trametinib for the treatment of patients with NSCLC whose tumors carry the proto-oncogene *BRAF* V600E mutation,[28] testing for this mutation could also be considered for SqCLC.[3] However, the mutation is rare in SqCLC and routine testing is therefore not recommended.[29, 30] Thus, currently, the vast majority of testing performed on SqCLC biopsy samples consists of p40/p63 immunostaining on tissue sections to confirm the histologic subtype and PD-L1 assessment to determine eligibility for checkpoint inhibition front-line.

The turnaround time for obtaining the results of molecular testing is an important concern, as patients with advanced disease benefit from starting appropriate treatment as soon as possible. The CAP/IASLC/AMP guidelines for clinical practice recommend a maximum of 2 weeks for the completion of all molecular testing.[1] A streamlined process that incorporates a multidisciplinary team is pivotal for meeting the benchmark turnaround time for the

completion of all molecular tests[31] (Figure 1). This process should include optimizing procedures and workflows, such as the transfer of tumor specimens between thoracic surgeons, interventional pulmonologists, radiologists, and pathologists and intra-laboratory communication. Recently, a study that analyzed routine nationwide molecular testing in France observed that obtaining results from molecular testing that approached acceptable turnaround times was feasible (median of 11 days from initiation of analysis to report of results).[32]

The type of assays used is also important, for which the CAP/IASLC/AMP guidelines further recommend that each laboratory determine the minimum proportion and number of cancer cells needed to detect a mutation during validation of an assay.[1] These guidelines were last published in 2013, and updated guidelines with evidence-based expert consensus opinion will be published soon.

Lastly, it is important to consider potential differences in the implementation of molecular testing for NSCLC, including SqCLC, which may affect successful adoption into practice. These differences may arise partly due to regional availability of tests, reimbursement policies, and treatment settings (e.g., community vs. academic centers).[32-34] Greater uniformity in the practical implementation of molecular testing for NSCLC may be achieved through the development of inter- and intra-institutional and network pathways.[32]

Technologies for Molecular Testing in NSCLC – Current and New Methods

In practice, the use of multiplex or next-generation sequencing (NGS) platforms for molecular testing is often restricted to larger academic centers; many community treatment settings still rely on single-gene testing or sending samples out to commercial laboratories for testing. For molecular testing of *EGFR* mutations in NSCLC, guidelines recommend the use of any validated methodology with adequate coverage of mutations in exons 18-21, including

mutations associated with specific drug resistance.[1-3, 35] The standard testing methodology for *ALK* gene rearrangements and *ROS-1* gene fusions is fluorescence in situ hybridization (FISH), but IHC with high-performance *ALK* antibodies is also an approved *ALK* assay used for treatment decisions.[1-3, 35, 36]

As additional therapeutic targets are identified and new treatments are approved for patients with SqCLC, moving toward prioritizing tissue preservation for molecular testing as standard procedure will become a major practical change for institutions and physicians who manage patients with this NSCLC subtype. The implementation of newer technologies, such as NGS, may assist in addressing the challenges associated with an increased need for performing molecular testing on small biopsy samples in SqCLC and improve turnaround times for molecular testing (Table 1). The current reality is, however, that the lack of genomic targets and approved therapies in SqCLC means that relatively few cases are subjected to molecular screening. Hence, tissue availability for PD-L1 IHC, for example, is therefore less challenging than for adenocarcinoma.

NGS. NGS technologies are high-throughput methods that allow for the parallel sequencing of multiple targeted genomic regions and include whole genome or exome capture sequencing (deoxyribonucleic acid-based sequencing platform), whole or targeted transcriptome sequencing (ribonucleic acid-based sequencing platform), and epigenetic profiling[37] (Table 1). The potential for increased clinical use of NGS is supported by the recent validation of an NGS-based framework as the primary molecular testing method in a large, prospective clinical trial with patients with advanced NSCLC.[38] As approved targeted treatments are limited for patients with SqCLC, routine molecular testing using NGS is not currently required. However, the use of NGS has facilitated the screening of patients for enrollment in ongoing clinical trials aimed at identifying new actionable molecular targets

and evaluating novel targeted therapies that may benefit this patient population.[39-42] NGS was also recently used in a study that showed that patients who had *ErbB*–mutation-positive SqCLC had higher progression-free survival (PFS) and overall survival when treated with afatinib than when treated with erlotinib, or with patients who had *ErbB*–mutation-negative disease.[43] These findings, in addition to the recent U.S. Food and Drug Administration (FDA) approval of an NGS-based companion test to identify patients with NSCLC eligible for treatment with crizotinib, gefitinib, and dabrafenib combined with trametinib, [44] support the clinical application of NGS for molecular testing in NSCLC, including SqCLC.

Furthermore, use of NGS for molecular testing in NSCLC may become routine with the potential role of tumor mutational burden (TMB) to assess the likelihood of benefit from immunotherapy. In a study that included 2 independent cohorts, patients with NSCLC whose tumors had a high TMB, or nonsynonymous mutation burden, experienced greater clinical benefit from treatment with the PD-1 inhibitor pembrolizumab than patients whose tumors had a lower mutation load.[45] More recently, results from a subset analysis of a phase III clinical trial showed that patients with NSCLC whose tumors had a high TMB and PD-L1 expression by IHC had a higher clinical response to first-line treatment with the PD-1 inhibitor nivolumab than with chemotherapy.[46]

Despite the applicability of NGS for molecular testing in NSCLC, and potentially for SqCLC as more targeted treatments become available, several drawbacks need to be addressed before it is routinely implemented in clinical practice. The implementation of NGS into regulatory and standard diagnostic pathways may be negatively affected by the multiple proprietary NGS variant databases,[47] the use of different methodologies (e.g., sequencing of non-amplified genome vs. amplicons),[48] the inconsistent concordance between different biopsy types such as liquid biopsies and matched tissue biopsies, and the very large volume of

complex bioinformatics data that require analysis.[49] Another potential drawback of NGS is the lack of uniform policy for supporting, covering, or reimbursing the use of NGS comprehensive molecular testing, presenting additional challenges to its implementation in clinical practice.[34, 47] Furthermore, many of the NGS platforms currently used in the clinical setting are amplicon-based, which do not detect gene fusions or gene rearrangements, unlike newer platforms such as Archer® (ArcherDX, Inc., Boulder, CO), FoundationOne® (Foundation Medicine, Cambridge, MA), and NovaSeq® (Illumina, Eindhoven, The Netherlands). Lastly, the limited information currently available on the applicability of NGS for biomarker testing relating to immunotherapies will further affect its adoption for molecular testing for SqCLC.

Analysis of Circulating-Tumor DNA (Liquid Biopsies). Liquid biopsies are performed on blood samples and can be used to assess circulating-tumor cells, circulating-tumor DNA, circulating cell-free DNA, and exosomes for tumor-associated genetic and molecular alterations through several approaches.[50, 51] The use of blood samples for liquid biopsies offers several potential advantages over tissue biopsy testing, including quick and non-invasive sample retrieval, faster testing turnaround times, and the potential for monitoring responses and resistance to treatment.[50, 51] Furthermore, NSCLC tumors are highly heterogeneous and the ability to assess circulating-tumor cells, circulating-tumor DNA, circulating cell-free DNA, and exosomes that derive from a patient's whole tumor or tumors allows for the detection of intra- and inter-tumor heterogeneity.[22, 50-53] In 2016, the FDA approved a companion diagnostic test for the detection of exon 19 deletions or exon 21 substitution mutations in *EGFR* from liquid biopsies to identify patients with NSCLC who were eligible for treatment with erlotinib.[54] The indication for the companion test was subsequently extended to include the detection of *EGFR* T790M mutations from liquid biopsies to identify tyrosine kinase inhibitor-resistant patients eligible for treatment with

osimertinib.[55] Despite recent advances, however, the remaining technical challenges, including inconsistent concordance compared with tissue,[50, 51, 56] will need to be overcome prior to the implementation of liquid biopsies into practice (Table 1).

Overall, a number of new technologies are becoming available for molecular testing and may assist in addressing some of the issues that will arise from an increased need for molecular testing in SqCLC in the near future. Validating these methodologies and using external quality assurance programs will be essential to ensuring accurate and timely results to guide treatment for patients.

Impact of New Treatments on Molecular and Immune Testing in SqCLC

Targeting genetic abnormalities in SqCLC remains a research aim; however, the molecular profile of SqCLC is complex and SqCLC tumors have a high mutation load.[22] Consequently, the profile of SqCLC is unlikely to offer many actionable molecular targets, as the dominant molecular changes are not addictive oncogenes.[22] Indeed, this lack of identifiable oncogenic drivers in SqCLC has proven to be a challenge, and targeting single genetic alterations seems to achieve only modest clinical benefits in advanced SqCLC.[57-62]

Conversely, the elucidation of how tumor cells employ various complex and overlapping mechanisms to evade the immune system[63] has led to an increased focus on immuno-oncology, particularly the PD-1/PD-L1 axis. Immunotherapy with anti–PD-1/–PD-L1 antibodies now provides an important alternative to chemotherapy for SqCLC.[64-67] The emergence of immunotherapy for the treatment of patients with advanced SqCLC has been transformative and will further impact the future of molecular and immune testing by leading to changes in the way genomic alterations are explored in SqCLC.

Given the challenges in developing targeted therapies for advanced SqCLC previously noted, novel study designs have been developed to evaluate additional potential targeted treatments for advanced SqCLC and, most recently, non-squamous NSCLC. For example, the Lung Cancer Master Protocol (Lung-MAP) study (SWOG S1400) seeks to identify potentially actionable molecular alterations in the second-line advanced SqCLC setting through the comprehensive screening of patients via an NGS platform.[39, 68] The NGS platform used in Lung-MAP detects base substitutions, short insertions and deletions, copy number alterations, and gene fusions across 287 cancer-related genes (Foundation Medicine, Cambridge, MA).[69] The rapid turnaround of results from the NGS screening (i.e., 10 to 14 days), which is critical for patients with advanced SqCLC, may be partly responsible for enabling patients to be prescreened with molecular testing prior to disease progression during or after first-line therapy, thus facilitating the efficient assignment of eligible patients to a sub-study based on the identification of biomarkers or to a non-match sub-study in which they receive immunotherapy. The testing approach of the Lung-MAP study may affect how new targeted agents are developed for SqCLC and non-squamous NSCLC and, consequently, may influence the implementation of additional molecular testing in practice.

Recently, 3 Lung-MAP phase II sub-studies that included the fibroblast growth factor receptor inhibitor AZD4547, the cyclin-dependent kinase 4/6 inhibitor palbociclib, and the phosphoinositide 3-kinase inhibitor taselisib failed to meet their primary end points in their respective biomarker-enriched cohorts of patients with SqCLC.[70-72] Nonetheless, the sub-studies served to catalog the array of diverse mutations present in these cancer-related genes among patients with SqCLC. On a rolling basis, new Lung-MAP sub-studies continue to be incorporated as new targeted therapies with actionable molecular targets become available.

Immune Biomarker Testing for Immunotherapy Treatments

Immune testing for checkpoint inhibitor PD-L1 protein expression as a predictive biomarker for response to anti–PD-1 or anti–PD-L1 antibodies is evolving. Testing for PD-L1 protein expression is performed by IHC, with each approved anti–PD-1/–PD-L1 immunotherapy having a different companion/complementary PD-L1 IHC assay.[73-78]

The anti–PD-1 agent pembrolizumab is approved for first-line treatment of patients with advanced NSCLC, including SqCLC, in patients with high PD-L1 expression (tumor proportion score ≥ 50%),[67, 75, 76] based on a phase III, prospective, randomized clinical study showing superior efficacy and lower toxicity for pembrolizumab than for chemotherapy.[67] Furthermore, second-line treatment of patients with advanced NSCLC with anti–PD-1 agents pembrolizumab and nivolumab and anti–PD-L1 agent atezolizumab have all demonstrated superiority to docetaxel chemotherapy after initial platinum doublet chemotherapy in randomized phase III studies.[64-66] The studies with nivolumab and atezolizumab included patients with any or no PD-L1 expression, while the study with pembrolizumab included only patients with a tumor proportion score of > 1%. However, the benefit of immunotherapy over chemotherapy increased with higher PD-L1 expression in each of these trials. Thus, PD-L1 testing at diagnosis for metastatic disease has been incorporated into guidelines such as the NCCN guidelines.[3] The recently updated American Society of Clinical Oncology treatment guidelines state that the guidance starts from the point at which the results of molecular and PD-L1 testing are known; however, reviewing the molecular testing literature is beyond the scope of the guideline.[79]

The existence of multiple distinct diagnostic assays for determining PD-L1 expression to guide treatment with each anti–PD-1/–PD-L1 antibody constitutes a barrier to routine implementation of PD-L1 testing in clinical practice due to the impracticality of conducting

multiple assays for the same protein. Consequently, there is great interest in establishing whether these assays provide comparable results for PD-L1 expression and could be used interchangeably in laboratories. Recently, comparison studies between the multiple PD-L1 assays reported a high degree of agreement between most assays.[80-82] However, interchanging the assays and PD-L1 expression cut-off values used for the different anti–PD-1/–PD-L1 antibodies led to a misclassified PD-L1 status for some patients, highlighting the need for standardization.[81] Validated cut-offs are a function of drug activity and should remain allied to the drug/indication relevant to the patient and not allied to the assay.

A further need for standardization of PD-L1 testing relates to the reporting of PD-L1 expression by pathologists. Identifying the subset of patients with NSCLC who will benefit the most from therapy with anti–PD-1/–PD-L1 antibodies can be challenging, given the diversity of PD-L1 expression levels used to stratify patients in clinical studies for different anti–PD-1/–PD-L1 antibodies.[46, 65, 67] Therefore, standardized pathology reporting for PD-L1 expression using a numeric value rather than stating PD-L1 positivity/negativity is mandatory for the treating oncologist.

More recently, a randomized phase II trial comparing pemetrexed and carboplatin plus pembrolizumab to pemetrexed and carboplatin in patients with non-squamous NSCLC showed superior results with respect to response rate and PFS for the combination with pembrolizumab.[83] Although the number of patients involved was small, there was some evidence that more patients with a PD-L1 tumor proportion score of ≥ 50% achieved an objective response with pembrolizumab plus chemotherapy (80%; n = 16) compared with patients with a tumor proportion score of 1-49% (26%; n = 5). While this study did not include patients with SqCLC, several randomized phase III trials in the first-line setting comparing treatment with anti–PD-1 and anti–PD-L1 checkpoint inhibitors alone or in

combination with anti-cytotoxic T–lymphocyte-associated protein 4 inhibitors and trials comparing chemotherapy alone or in combination with checkpoint inhibitors are currently ongoing. The results of these trials will undoubtedly determine the role of immune testing for PD-L1 protein expression at diagnosis, depending on where the role of first-line immunotherapy is challenged. In addition, a recent randomized phase III trial in patients with stage III NCSLC, including SqCLC, showed that standard chemotherapy/radiotherapy followed by durvalumab yielded superior PFS compared to chemotherapy/radiotherapy alone, irrespective of PD-L1 expression before chemotherapy/radiotherapy.[84] Other trials with checkpoint inhibitors are ongoing in patients with stage III NSCLC and in the adjuvant setting in patients with earlier-stage disease. The results of these trials may influence how we test for PD-L1 expression in these stages, but for now, we suggest a pathway for this testing in Figure 2.

Because PD-L1 protein expression is an imperfect biomarker, other potential biomarkers such as TMB are currently being evaluated in several ongoing studies. At present, assessment of TMB is not standardized and it is not part of routine management. However, recent retrospective studies showing that high TMB predicted favorable outcomes for checkpoint inhibitor therapy and that the combination of TMB with PD-L1 expression levels was superior to either marker alone[45] support the implementation of TMB for use as a biomarker in the future.

Discussion: The Future for Molecular and Immune Testing in SqCLC

The molecular and immune testing landscape for SqCLC is likely to change rapidly over the next several years due to the emergence of immunotherapies such as anti–PD-L1 and anti–PD-1 antibodies and novel targeted therapies for advanced SqCLC. Indeed, the need to test for PD-L1 expression levels before prescribing pembrolizumab as first-line therapy for

advanced NSCLC, including SqCLC, has already meant that institutions are beginning to implement this test as part of standard practice. In some instances, this is occurring "reflexively," without requiring additional orders. Therefore, integration of new molecular and immune testing into standard diagnostic and treatment algorithms and guidelines for advanced SqCLC will become essential to ensuring that patients receive appropriate and timely treatment.

Initially, the use of NGS for molecular testing in SqCLC is more likely to be adopted over other testing platforms due to features such as tumor tissue sample optimization, fast turnaround, and comprehensive genomic testing. The use of NGS testing may further expand as the significance of TMB as a biomarker for response to immunotherapy becomes better understood. However, analyses on value (in clinical trials) and the cost of increased screening and the use of comprehensive technology platforms that test for more than standard genetic alterations with approved targeted therapies will be necessary for these platforms to be widely accepted among payers and regulators.

Lastly, as molecular testing for SqCLC evolves, greater education for patients will be needed. Improved patient communication will help patients understand the need for, timing of, eligibility for, and results from molecular tests and how these results may affect their treatment options.

Conclusion

The workload for pathologists will increase due to increased requests for genomic and proteomic profiles in SqCLC. The establishment of multidisciplinary teams and best practices for institutions to accommodate the need for, and to meet benchmark timelines for, molecular and immune biomarker testing for NSCLC, including, SqCLC, is recommended. Furthermore, as new therapeutic targets are identified for SqCLC, standardized pathology

reporting of new genomic and proteomic test results will play an important role in ensuring that accurate, concise, and appropriate information is available for clinicians to guide treatment decisions.

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Table 1 Key Features of Single-Gene, Next-Generation Sequencing, and Liquid Biopsy Technologies in SqCLC[39-42, 50, 51, 85]

Technology	Single-Gene Testing	Next-Generation Sequencing	Liquid Biopsy
Features	• Targeted gene testing using Sanger DNA sequencing, RT-PCR, FISH, and IHC	• High-throughput genetic profiling for decision-making in individual patients • Includes whole genome or exome capture sequencing of DNA, whole or targeted transcriptome sequencing of RNA, and epigenetic profiling	• Analysis of circulating cell-free DNA from plasma via quick and non-invasive retrieval • Method for potentially monitoring responses and resistance to treatment
Advantages for SqCLC	• Current approach for decision-making in individual patients if it can be performed in the benchmark turnaround time for results	• Allows for the sparing of limited SqCLC tumor tissue for testing • Expands testing beyond currently known biomarkers • Facilitates the screening of patients with SqCLC for enrollment in ongoing clinical trials aimed at identifying new actionable molecular targets	• May allow for an initial diagnosis of patients who may not be able to undergo a biopsy due to advanced disease • Analyzes circulating-tumor cells, circulating-tumor DNA, circulating cell-free DNA, and exomes, which may help overcome sampling and tumor heterogeneity
Limitations for SqCLC	• Tissue samples are often inadequate for all required testing, requiring greater tissue prioritization	• Multiple proprietary databases negatively impact implementation into regulatory and	• Testing of circulating-tumor cells is not yet optimized for use with next-generation

		standard diagnostic pathways • Potential issues with reimbursement may affect implementation of comprehensive molecular testing into clinical practice • Most NGS platforms used in clinical institutions are amplicon-based, which do not detect gene fusions or rearrangements • Analysis of a large volume of bioinformatics • Limited information on its applicability for biomarker testing relating to immunotherapies	sequencing and other less sensitive platforms • Technical challenges remain to validate and implement for use in clinical practice

Abbreviations: DNA = deoxyribonucleic acid; FISH = fluorescent in situ hybridization; IHC = immunohistochemistry; NGS = next-generation sequencing; RNA = ribonucleic acid; RT-PCR = reverse transcription polymerase chain reaction; SqCLC = squamous-cell lung cancer.

Figure 1 Multidisciplinary Scheme and Best Practice Timelines for Each Clinical Stage Following the Patient's Referral

Abbreviations: *ALK* = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; FISH = fluorescent in situ hybridization; IHC = immunohistochemistry; MDT = multidisciplinary team; PD-L1 = programmed death-ligand 1; *ROS-1* = *ROS* proto-oncogene 1.

Figure 2 Recommended Molecular and Immune Biomarker Testing for Patients With Confirmed SqCLC Histology

Abbreviations: *ALK* = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; FISH = fluorescent in situ hybridization; IHC = immunohistochemistry; PD-L1 = programmed death-ligand 1; *ROS-1* = *ROS* proto-oncogene 1; SqCLC = squamous-cell lung cancer.